STN

FILE 'HOME' ENTERED AT 18:02:41 ON 23 FEB 2005

L1 QUE (G-CSF OR GCSF OR HG-CSF OR (GRANULOCYTE-COLONY (A) STIMULATING OR GRANULOCYTE (A) (COLONY-STIMULATING OR COLONY (A) STIMULATING))) (A) FACTOR AND (MUTANT OR VARIANT OR ANALOG### OR SUBSTITUTION OR MODIFIC? OR PEG OR POLYETHYLENE)

L3 6003 (G-CSF OR GCSF OR HG-CSF OR (GRANULOCYTE-COLONY (A) STIMULATING OR GRANULOCYTE (A) (COLONY-STIMULATING OR COLONY (A) STIMULATING) (A) FACTOR)) AND (MUTANT OR VARIANT OR ANALOG### OR SUBSTITUTION OR MODIFIC? OR PEG OR POLYETHYLENE)

L4 505 (G-CSF OR GCSF OR HG-CSF OR (GRANULOCYTE-COLONY (A) STIMULATING OR GRANULOCYTE (A) (COLONY-STIMULATING OR COLONY (A) STIMULATING) (A) FACTOR)) (P) (PEG OR POLYETHYLENE)

L7 2259 L3 AND (G-CSF OR GCSF OR HG-CSF OR (GRANULOCYTE-COLONY (A) STIMULATING OR GRANULOCYTE (A) (COLONY-STIMULATING OR COLONY (A) STIMULATING) (A) FACTOR)) (S) (MUTANT OR VARIANT OR ANALOG### OR SUBSTITUTION OR MODIFIC?)

L8 17 L7 AND (LYSINE OR GLUTAMINE) (S) (SUBSTITUT? OR MODIF? OR ATTACH? OR POLYETHYLENE OR PEG OR POLYMER)

L10 1641 L7 AND (G-CSF OR GCSF OR HG-CSF OR (GRANULOCYTE-COLONY (A) STIMULATING OR GRANULOCYTE (A) (COLONY-STIMULATING OR COLONY (A) STIMULATING) (A) FACTOR)) (S) (MUTANT OR ANALOG### OR SUBSTITUT?)

L13 1868 L7 AND (G-CSF OR GCSF OR HG-CSF OR "GRANULOCYTE-COLONY STIMULATING" OR "GRANULOCYTE COLONY-STIMULATING" OR "GRANULOCYTE COLONY STIMULATING")/AB

(FILE 'HOME' ENTERED AT 18:02:41 ON 23 FEB 2005)

INDEX 'ADISCTI, ADISINSIGHT, ADISNEWS, AGRICOLA, ANABSTR, ANTE, AQUALINE, AQUASCI, BIOBUSINESS, BIOCOMMERCE, BIOENG, BIOSIS, BIOTECHABS, BIOTECHDS, BIOTECHNO, CABA, CANCERLIT, CAPLUS, CEABA-VTB, CEN, CIN, CONFSCI, CROPB, CROPU, DDFB, DDFU, DGENE, DISSABS, ...' ENTERED AT 18:03:03 ON 23 FEB 2005

SEA (G-CSF OR GCSF OR HG-CSF OR (GRANULOCYTE-COLONY (A) STIMULA

94	FILE ADISCTI
21	FILE ADISINSIGHT
28	FILE ADISNEWS
3	FILE AGRICOLA
3	FILE ANABSTR
15	FILE BIOBUSINESS
10	FILE BIOCOMMERCE
29	FILE BIOENG
587	FILE BIOSIS
140	FILE BIOTECHABS
140	FILE BIOTECHDS
526	FILE BIOTECHNO
8	FILE CABA
892	FILE CANCERLIT
626	FILE CAPLUS
13	FILE CEABA-VTB
1	FILE CEN
5	FILE CIN

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              2   FILE CONFSCI
            107   FILE DDFU
           1183   FILE DGENE
              7   FILE DISSABS
            199   FILE DRUGU
              3   FILE EMBAL
            947   FILE EMBASE
            240   FILE ESBIOBASE
             11   FILE FEDRIP
              1   FILE HEALSAFE
            250   FILE IFIPAT
             30   FILE IMSDRUGNEWS
              9   FILE IMSRESEARCH
             55   FILE JICST-EPLUS
            122   FILE LIFESCI
           1092   FILE MEDLINE
              1   FILE NTIS
            269   FILE PASCAL
              8   FILE PHAR
              3   FILE PHARMAML
              8   FILE PHIN
             84   FILE PROMT
              4   FILE PROUSDDR
            408   FILE SCISEARCH
            979   FILE TOXCENTER
           4433   FILE USPATFULL
            279   FILE USPAT2
              2   FILE VETU
            241   FILE WPIDS
              1   FILE WPIFV
            241   FILE WPINDEX
L1             QUE (G-CSF OR GCSF OR HG-CSF OR (GRANULOCYTE-COLONY (A) STIMULA
               ---------

     FILE 'MEDLINE, CAPLUS, BIOSIS, EMBASE, SCISEARCH, BIOTECHNO, CANCERLIT'
     ENTERED AT 18:10:13 ON 23 FEB 2005
L2          5078 S L1
L3          6003 S (G-CSF OR GCSF OR HG-CSF OR (GRANULOCYTE-COLONY (A) STIMULATI
L4           505 S (G-CSF OR GCSF OR HG-CSF OR (GRANULOCYTE-COLONY (A) STIMULATI
L5           212 DUP REM L4 (293 DUPLICATES REMOVED)
L6           108 S L5 AND PY<2001
L7          2259 S L3 AND  (G-CSF OR GCSF OR HG-CSF OR (GRANULOCYTE-COLONY (A)
L8            17 S L7 AND (LYSINE OR GLUTAMINE ) (S) (SUBSTITUT? OR MODIF? OR A
L9             9 DUP REM L8 (8 DUPLICATES REMOVED)
L10         1641 S L7 AND  (G-CSF OR GCSF OR HG-CSF OR (GRANULOCYTE-COLONY (A)
L11            0 S L7 AND  (G-CSF OR GCSF OR HG-CSF OR (GRANULOCYTE-COLONY (A)
L12            0 S L7 AND  (G-CSF OR GCSF OR HG-CSF OR "GRANULOCYTE-COLONY STIM
L13         1868 S L7 AND  (G-CSF OR GCSF OR HG-CSF OR "GRANULOCYTE-COLONY STIM
L14          726 DUP REM L13 (1142 DUPLICATES REMOVED)
L15          511 S L14 AND L10
L16          320 S L15 AND PY<2000
L17            6 S L16 AND L5
L18            6 S L17 NOT L9
```

L9 ANSWER 2 OF 9 CAPLUS COPYRIGHT 2005 ACS on STN
AN 2001:526095 CAPLUS
DN 135:127157
TI **Granulocyte colony-stimulating factor (G-CSF)** conjugates for therapeutic uses
IN Nissen, Torben Lauesgaard; Andersen, Kim Vilbour; Hansen, Christian Karsten; Mikkelsen, Jan Moller; Schambye, Hans Thalsgaard
PA Maxygen Aps, Den.
SO PCT Int. Appl., 94 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 4

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 2001051510	A2	20010719	WO 2001-DK11	20010109
	WO 2001051510	A3	20020321		
	W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
	RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
	CA 2395713	AA	20010719	CA 2001-2395713	20010109
	EP 1250154	A2	20021023	EP 2001-900105	20010109
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR				
	BR 2001007561	A	20021119	BR 2001-7561	20010109
	JP 2003519478	T2	20030624	JP 2001-551094	20010109
	NZ 520261	A	20031031	NZ 2001-520261	20010109
	ZA 2002004623	A	20021211	ZA 2002-4623	20020610
	NO 2002003315	A	20020905	NO 2002-3315	20020709
PRAI	DK 2000-24	A	20000110		
	DK 2000-341	A	20000302		
	DK 2000-943	A	20000616		
	WO 2001-DK11	W	20010109		

AB The invention relates to polypeptide conjugates comprising a polypeptide exhibiting **G-CSF** activity and having an amino acid sequence that differs from the amino acid sequence of human **G-CSF** in at least one specified introduced and/or removed amino acid residue comprising an attachment group for a non-polypeptide moiety, and having at least one non-polypeptide moiety attached to an attachment group of the polypeptide. The **attachment** group may e.g. be a **lysine**, cysteine, aspartic acid or glutamic acid residue or a glycosylation site, and the non-polypeptide moiety may e.g. be a **polymer** such as **polyethylene** glycol or an oligosaccharide. The conjugate has one or more improved properties such as increased biol. half-life and reduced side effects.

5 OF 9 CAPLUS COPYRIGHT 2005 ACS on STN
AN 1997:116494 CAPLUS
DN 126:113153
TI **Modification** of polypeptide drugs to increase electrotransport flux
IN Holladay, Leslie A.
PA Alza Corporation, USA
SO PCT Int. Appl., 32 pp.

CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9639422	A2	19961212	WO 1996-US9377	19960606
	WO 9639422	A3	19970306		
	W: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG				
	RW: KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN				
	NL 1003283	A1	19961209	NL 1996-1003283	19960606
	NL 1003283	C2	19970502		
	CA 2220146	AA	19961212	CA 1996-2220146	19960606
	FR 2735132	A1	19961213	FR 1996-7002	19960606
	FR 2735132	B1	19980424		
	AU 9665903	A1	19961224	AU 1996-65903	19960606
	BE 1009704	A3	19970701	BE 1996-517	19960606
	GB 2317179	A1	19980318	GB 1997-25981	19960606
	GB 2317179	B2	19990728		
	DE 19681439	T	19980723	DE 1996-19681439	19960606
	BR 9609149	A	19990223	BR 1996-9149	19960606
	JP 11507341	T2	19990629	JP 1996-501710	19960606
	US 2002107505	A1	20020808	US 2001-16403	20011210
PRAI	US 1995-466610	A	19950606		
	WO 1996-US9377	W	19960606		

AB Methods of modifying polypeptide drugs in order to enhance their transdermal electrotransport flux are provided. The polypeptide is **modified** by **substituting** a histidine residue (His) for one or more **glutamine** (Gln), threonine (Thr) and/or asparagine (Asn) residue(s). The His for Gln **substitution** is particularly preferred from the standpoint of retaining biol. activity of the parent polypeptide. Compns. containing the modified polypeptide, which are useful for transdermal electrotransport delivery, are also provided. **Analogs**, e.g. a PTH **analog**, showed improved electrotransport plasma levels. A schematic drawing of an electrotransport drug delivery device is included.

L9 ANSWER 6 OF 9 CAPLUS COPYRIGHT 2005 ACS on STN
AN 1995:615193 CAPLUS
DN 123:25669
TI Peptides derived from hemopoietic growth factors as antagonists of the growth factors
IN Vadas, Mathew Alexander; Lopez, Angel Francisco; Shannon, Mary Frances
PA Medvet Science Pty. Ltd., Australia
SO PCT Int. Appl., 60 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 3

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9504075	A1	19950209	WO 1994-AU432	19940728
	W: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, ES, FI, GB, GE, HU, JP, KE, KG, KP, KR, KZ, LK, LT, LU, LV, MD, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SI, SK, TJ, TT, UA, US, UZ, VN				
	RW: KE, MW, SD, AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				

	CA 2168261	AA	19950209	CA 1994-2168261	19940728
	AU 9473414	A1	19950228	AU 1994-73414	19940728
	AU 690128	B2	19980423		
	EP 715633	A1	19960612	EP 1994-922181	19940728
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE				
	JP 09501154	T2	19970204	JP 1994-505450	19940728
	US 5939063	A	19990817	US 1996-591438	19960408
	NZ 329156	A	20000728	NZ 1997-329156	19971111
	AU 9934974	A1	19990909	AU 1999-34974	19990611
PRAI	AU 1993-186	A	19930728		
	AU 1994-4772	A	19940330		
	WO 1994-AU432	W	19940728		
	AU 1996-61153	A3	19960621		
	NZ 1997-269766	A1	19971111		

AB Modified and **variant** forms of hemopoietic growth factors (HGF) capable of acting as antagonists to the corresponding native hemopoietic growth factors are described for use in ameliorating aberrant effects caused by the native mols. A modified hemopoietic growth factor (HGF) is characterized by being in unglycosidated form and has an α-helical domain with one or more of any exposed acidic amino acids substituted with a basic amino acid. The preferred HGF are granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukins (IL)-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-9, IL-10, **G-CSF** and erythropoietin (EPO). The synthesis and biol. activity of a number of such peptides is demonstrated.

L9 ANSWER 7 OF 9 MEDLINE on STN DUPLICATE 2
AN 95349657 MEDLINE
DN PubMed ID: 7542747
TI Mutations in the gene for the **granulocyte colony-stimulating-factor** receptor in patients with acute myeloid leukemia preceded by severe congenital neutropenia.
CM Comment in: N Engl J Med. 1995 Aug 24;333(8):516-8. PubMed ID: 7542748
AU Dong F; Brynes R K; Tidow N; Welte K; Lowenberg B; Touw I P
CS Department of Hematology, Dr. Daniel den Hoed Cancer Center, Rotterdam, The Netherlands.
SO New England journal of medicine, (1995 Aug 24) 333 (8) 487-93. Journal code: 0255562. ISSN: 0028-4793.
CY United States
DT (CASE REPORTS)
Journal; Article; (JOURNAL ARTICLE)
LA English
FS Abridged Index Medicus Journals; Priority Journals
OS GENBANK-S78382; GENBANK-S78385
EM 199508
ED Entered STN: 19950911
Last Updated on STN: 19960129
Entered Medline: 19950830
AB BACKGROUND. In severe congenital neutropenia the maturation of myeloid progenitor cells is arrested. The myelodysplastic syndrome and acute myeloid leukemia develop in some patients with severe congenital neutropenia. Abnormalities in the signal-transduction pathways for **granulocyte colony-stimulating factor** (**G-CSF**) may play a part in the progression to acute myeloid leukemia. METHODS. We isolated genomic DNA and RNA from hematopoietic cells obtained from two patients with acute myeloid leukemia and histories of severe congenital neutropenia. The nucleotide sequences encoding the cytoplasmic domain of the **G-CSF** receptor were amplified by means of the polymerase chain reaction and sequenced. Murine myeloid 32D.C10 cells were transfected with complementary DNA encoding the wild-type or **mutant G-CSF**

receptors and tested for their responses to **G-CSF**. RESULTS. Point mutations in the gene for the **G-CSF** receptor were identified in both patients. The mutations, a **substitution** of thymine for cytosine at the codon for **glutamine** at position 718 (Gln718) in one patient and at the codon for **glutamine** at position 731(Gln731) in the other, caused a truncation of the C-terminal cytoplasmic region of the receptor. Both **mutant** and wild-type genes for the **G-CSF** receptor were present in leukemic cells from the two patients. In one patient, the mutation was also found in the neutropenic stage, before the progression to acute myeloid leukemia. The 32D.C10 cells expressing **mutant** receptors had abnormally high proliferative responses but failed to mature when cultured in **G-CSF**. The **mutant G-CSF** receptors also interfered with terminal maturation mediated by the wild-type **G-CSF** receptor in the 32D.C10 cells that coexpressed the wild-type and **mutant** receptors. CONCLUSIONS. Mutations in the gene for the **G-CSF** receptor that interrupt signals required for the maturation of myeloid cells are involved in the pathogenesis of severe congenital neutropenia and associated with the progression to acute myeloid leukemia.

L9 ANSWER 8 OF 9 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation. on STN DUPLICATE 3

AN 92:347820 SCISEARCH

GA The Genuine Article (R) Number: HX055

TI CONSTRUCTION OF PROTEIN **ANALOGS** BY SITE-SPECIFIC CONDENSATION OF UNPROTECTED FRAGMENTS

AU GAERTNER H F (Reprint); ROSE K; COTTON R; TIMMS D; CAMBLE R; OFFORD R E

CS UNIV GENEVA, CTR MED, DEPT BIOCHIM MED, CTR 1 RUE MICHEL SERVET, CH-1211 GENEVA 4, SWITZERLAND (Reprint); ICI PHARMACEUT PLC, MACCLESFIELD, CHESHIRE, ENGLAND

CYA SWITZERLAND; ENGLAND

SO BIOCONJUGATE CHEMISTRY, (MAY/JUN 1992) Vol. 3, No. 3, pp. 262-268. ISSN: 1043-1802.

DT Article; Journal

FS LIFE

LA ENGLISH

REC Reference Count: 34

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The extreme sensitivity to periodate of 1-amino, 2-hydroxy compounds permits the selective conversion of N-terminal serine and threonine to an aldehydic group. We have used this reaction to construct **analogues** of human **granulocyte colony stimulating factor** (**G-CSF**) by allowing such oxidized peptides to react with others that have had a hydrazide derivative **attached** to the C-terminus by reversed proteolysis. Two recombinant **analogues** of **G-CSF** were used as starting materials. Both had only a single **lysine** residue (at position 62 and 75, respectively) followed immediately by a serine. Digestion of each **analogue** by the **lysine**-specific protease from Achromobacter lyticus gave two fragments, one of which could be N-terminally oxidized and the other converted to the C-terminal hydrazide derivative by reversed proteolysis using the same enzyme. After preliminary studies with model peptides, we first reacted the corresponding peptide pairs together and then, in order to eliminate the 64-74 disulfide loop, fragment 1-62 from the first **analogue** with fragment 76-174 from the second. Reactions are efficient (up to 80 % product based on the oxidized fragment) and take place under very mild conditions. The hydrazone bond can easily be stabilized by reduction with NaBH3CN. This method represents a new, reasonably general route for the

construction of large protein chimeras of precisely controlled structure.

L9 ANSWER 9 OF 9 CAPLUS COPYRIGHT 2005 ACS on STN
AN 1990:401547 CAPLUS
DN 113:1547
TI Site-specific homogeneous **modification** of polypeptides to facilitate covalent linkages to a hydrophilic moiety
IN Shaw, Gray
PA Genetics Institute, Inc., USA
SO PCT Int. Appl., 37 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 8905824	A1	19890629	WO 1988-US4633	19881222
	W: AU, JP				
	RW: AT, BE, CH, DE, FR, GB, IT, LU, NL, SE				
	US 4904584	A	19900227	US 1987-137043	19871223
	AU 8929111	A1	19890719	AU 1989-29111	19881222
	EP 355142	A1	19900228	EP 1989-901043	19881222
	R: AT, BE, CH, DE, FR, GB, IT, LI, LU, NL, SE				
	JP 02502646	T2	19900823	JP 1989-500925	19881222
PRAI	US 1987-137043	A	19871223		
	WO 1988-US4633	A	19881222		

AB To improve the homogeneity of chemical **modification** of a protein by a hydrophilic moiety e.g. **polyethylene** glycol, the number of potentially reactive **lysines** on the surface of the protein is changed by site-directed mutagenesis of the cloned gene. **Lysines** are **substituted** with or for arginine as necessary. An Arg16, Arg34, Lys147 derivative of **granulocyte colony stimulating factor** was prepared by oligonucleotide-directed site-specific mutagenesis of the cloned gene in the plasmid pxMT2G-CSF. After expression of the altered gene in animal cells the protein may be conjugated with **polyethylene** glycol by standard methods.

L18 ANSWER 1 OF 6 CAPLUS COPYRIGHT 2005 ACS on STN
AN 1998:806675 CAPLUS
DN 130:66807
TI Preparation of chemically modified polypeptides for treatment of patients with reduced counts of granulocyte or blood platelet
IN Yamasaki, Motoo; Suzawa, Toshiyuki; Kobayashi, Ken; Konishi, Noboru; Akinaga, Shiro; Maruyama, Kumiko
PA Kyowa Hakko Kogyo Co., Ltd., Japan
SO PCT Int. Appl., 44 pp.
CODEN: PIXXD2
DT Patent
LA Japanese
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9855500	A1	19981210	WO 1998-JP2504	19980605 <--
	W: AU, BG, BR, CA, CN, CZ, HU, IL, JP, KR, MX, NO, NZ, PL, RO, SG, SI, SK, UA, US, VN, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
	RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
	CA 2263795	AA	19981210	CA 1998-2263795	19980605 <--
	AU 9875512	A1	19981221	AU 1998-75512	19980605 <--
	AU 744085	B2	20020214		
	EP 921131	A1	19990609	EP 1998-923147	19980605 <--
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
	NZ 334068	A	20000728	NZ 1998-334068	19980605
	US 2002028912	A1	20020307	US 1999-230733	19990203
	US 6583267	B2	20030624		
	NO 9900560	A	19990326	NO 1999-560	19990205 <--
	US 2003195339	A1	20031016	US 2003-365418	20030213
PRAI	JP 1997-149342	A	19970606		
	WO 1998-JP2504	W	19980605		
	US 1999-230733	A3	19990203		

AB Claimed are chemical modified polypeptides, in particular having **granulocyte colony stimulating factor** activity, wherein at least one of the hydroxyl groups of a polypeptide mol. has been modified with polyalkylene glycols; a process for producing these polypeptides; a therapeutic method for treating patients with reduced counts of granulocyte or blood platelet by the use of these polypeptides; and therapeutic compns. containing these polypeptides. Thus, 205.5 mg monomethoxypolyethylene glycol propionic acid N-hydroxysuccinimide ester (M-SPA-20,000, Shearwater Polymer Corp.) was added to a 4.6 mg/mL solution of human **granulocyte colony stimulating factor** (**hG-CSF**) **analog**, i.e. [Thr1, Leu3, Tyr4, Arg5, Ser17]-Met-**hG-CSF**, in a phosphate buffer (pH 7.5) and stirred at 4° overnight to give **polyethylene** glycol-modified **hG-CSF** derivs. which were purified by a chromatog. Sephacryl S-400 column to give two mono-, one di-, and two tri(**polyethylene** glycol) derivs. of **hG-CSF**. The linkage positions of **polyethylene** glycol in the polypeptide were investigated by peptide mapping using V8 protease digestion and HPLC separation and mass spectroscopy of the peptide fragments for these mono(**polyethylene** glycol) derivs. In two mono(**polyethylene** glycol) derivs. isolated, **polyethylene** glycol was linked to N-terminal Met and the hydroxy group of serine at 66 position, resp. Mono- and di(**polyethylene** glycol) derivs. showed the enhancement of proliferation of NFS60 cells equal to that of **hG-CSF** **analog**. The mono(**polyethylene** glycol) derivative linked to

the Ser66 was 1.06-1.13 times more active than one linked to the terminal Met for enhancing the proliferation of NFS60 cells and was more stable in freezing-melting cycle test and more stable to thermolysin hydrolysis than the latter derivative

RE.CNT 11 THERE ARE 11 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L18 ANSWER 2 OF 6 CAPLUS COPYRIGHT 2005 ACS on STN
AN 1997:9226 CAPLUS
DN 126:27299
TI Recombinant preparation of fusion protein consisting of human thrombopoietin and **G-CSF** for treating anemia
IN Yokoi, Haruhiko; Shiotsu, Yukimasa; Konishi, Noboru; Anazawa, Hideharu; Tamaoki, Tatsuya; Yamasaki, Motoo; Terasaki, Yoko; Uchida, Kazuhisa; Yamashita, Kinya
PA Kyowa Hakko Kogyo Co., Ltd., Japan
SO PCT Int. Appl., 73 pp.
CODEN: PIXXD2
DT Patent
LA Japanese
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9634016	A1	19961031	WO 1996-JP1157	19960426 <--
	W: AU, CA, JP, KR, US				
	RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
	CA 2194070	AA	19961031	CA 1996-2194070	19960426 <--
	AU 9655147	A1	19961118	AU 1996-55147	19960426 <--
	AU 705064	B2	19990513		
	EP 783003	A1	19970709	EP 1996-912262	19960426 <--
	R: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE				
PRAI	JP 1995-102625	A	19950426		
	WO 1996-JP1157	W	19960426		

AB A method for recombinant preparation of fusion proteins consisting of human thrombopoietin (TPO) and a **G-CSF** derivative (ND28) by expression of their chimeric gene in animal cells was demonstrated. The fusion protein may contain a peptide linker. The fusion protein may be further modified with a polyalkylene glycol such as **polyethylene** glycol. Therapeutics for treating anemia containing the fusion proteins are claimed.

L18 ANSWER 3 OF 6 CAPLUS COPYRIGHT 2005 ACS on STN
AN 1996:398572 CAPLUS
DN 125:95821
TI Engineering **G-CSF** for improved depot formulation
AU Camble, Roger
CS ZENECA Pharmaceuticals, Macclesfield/Cheshire, SK10 4TG, UK
SO Perspectives on Protein Engineering & Complementary Technologies, Collected Papers, International Symposium, 3rd, Oxford, Sept. 13-17, 1994 (**1995**), Meeting Date 1994, 193-196. Editor(s): Geisow, Michael J.; Epton, Roger. Publisher: Mayflower Worldwide, Kingswinford, UK.
CODEN: 62ZQAP
DT Conference
LA English
AB The objective was to identify a **G-CSF** derivative compatible with continuous release from polylactide-co-glycolide copolymers similar to those used for the Zoladex depot. **Substitutions** designed to increase surface hydrophilicity or conformational stability were made in the amino acid sequence and highly potent **analogs** identified with improved solution stability at high

protein concentration Chemical **modification** of **analogs** by reaction with a large excess of activated monomethyl **polyethylene** glycol provided **G-CSF** derivs. with the desired profile of release from depot formulations.

L18 ANSWER 4 OF 6 CAPLUS COPYRIGHT 2005 ACS on STN
AN 1993:618355 CAPLUS
DN 119:218355
TI Polypeptide derivatives of human **granulocyte colony-stimulating factor** (**hG-CSF**)
IN Kuga, Tetsuro; Miyaji, Hiromasa; Sato, Moriyuki; Okabe, Masami; Morimoto, Makoto; Itoh, Seiga; Yamasaki, Motoo; Yokoo, Yoshiharu; Yamaguchi, Kazuo; et al.
PA Kyowa Hakko Kogyo Co., Ltd., Japan
SO U.S., 58 pp. Cont.-in-part of U.S. Ser. No. 318,527.
CODEN: USXXAM
DT Patent
LA English
FAN.CNT 5

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE	
PI	US 5214132	A	19930525	US 1989-337002	19890412	<--
	JP 10052281	A2	19980224	JP 1997-114630	19871223	<--
	JP 01225495	A2	19890908	JP 1988-51357	19880304	<--
	US 5194592	A	19930316	US 1989-318527	19890303	<--
	US 5362853	A	19941108	US 1992-994924	19921222	<--
	US 6027720	A	20000222	US 1994-274433	19940713	
	US 5681720	A	19971028	US 1995-434411	19950503	<--
	US 5714581	A	19980203	US 1995-434402	19950503	<--
	US 5795968	A	19980818	US 1997-783288	19970110	<--
	US 5994518	A	19991130	US 1997-890640	19970709	<--
PRAI	JP 1986-306799	A	19861223			
	US 1987-136647	B2	19871222			
	JP 1988-51357	A	19880304			
	JP 1988-80088	A	19880331			
	US 1989-318527	A2	19890303			
	JP 1994-185787	A3	19871223			
	US 1989-337002	A3	19890412			
	US 1992-994924	A3	19921222			
	US 1994-274433	A3	19940713			
	US 1995-434411	A3	19950503			

AB **HG-CSF**-derived polypeptides with different amino acid **substitutions** in the N-terminal region of **hG-CSF** are prepared by recombinant methods and enzyme cleavage. **Mutant hG-CSF** with Ala-1, Thr-3, Tyr-4, Arg-5, and Ser-17 (I) is claimed. I and **hG-CSF** were chemical modified with **PEG** derivs. to make products with enhanced peripheral leukocyte (granulocyte)-increasing effect and improved stability and residence time in the blood.

L18 ANSWER 5 OF 6 CAPLUS COPYRIGHT 2005 ACS on STN
AN 1992:639827 CAPLUS
DN 117:239827
TI Polypeptide-polymer conjugate continuous-release pharmaceutical compositions
IN Camble, Roger; Timms, David; Wilkinson, Anthony James
PA Imperial Chemical Industries PLC, UK
SO Brit. UK Pat. Appl., 206 pp.
CODEN: BAXXDU
DT Patent
LA English

FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	GB 2246295	A1	19920129	GB 1991-15207	19910715 <--
	GB 2246295	B2	19940511		
	FI 9103410	A	19920124	FI 1991-3410	19910715 <--
	EP 473268	A2	19920304	EP 1991-306452	19910716 <--
	EP 473268	A3	19920916		
	EP 473268	B1	20031008		
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE				
	ZA 9105555	A	19920429	ZA 1991-5555	19910716 <--
	AT 251641	E	20031015	AT 1991-306452	19910716
	CA 2047540	AA	19920124	CA 1991-2047540	19910722 <--
	AU 9181238	A1	19920130	AU 1991-81238	19910722 <--
	AU 655187	B2	19941208		
	HU 60632	A2	19921028	HU 1991-2442	19910722 <--
	JP 05032559	A2	19930209	JP 1991-271743	19910722 <--
	JP 3188292	B2	20010716		
	US 5320840	A	19940614	US 1991-734225	19910722 <--
	US 5773581	A	19980630	US 1995-488457	19950607 <--
PRAI	GB 1990-16138	A	19900723		
	GB 1990-18414	A	19900823		
	GB 1990-18415	A	19900823		
	GB 1990-18416	A	19900823		
	GB 1990-18417	A	19900823		
	GB 1990-18418	A	19900823		
	US 1991-734225	A3	19910722		
	US 1993-155327	B1	19931122		

AB Pharmaceutical compns. for continuous release of an acid stable physiol. active substance (polypeptide) from material of the composition (e.g. polylactide or biodegradable hydrogel) into an aqueous physiol.-type environment, comprise a polypeptide covalently conjugated to a water soluble polymer and incorporated into a matrix of polylactide, etc.; the polypeptide is released over a period of ≥1 wk. Human **granulocyte colony-stimulating factor** (**hG-CSF**) and solution-stable derivs. thereof were prepared by recombinant DNA methods and conjugated with Me **PEGs**. Continuous-release pharmaceutical compns. contained the conjugates incorporated in polylactide (50 weight% D,L-lactide/50 weight% glycolide copolymer) matrix.

L18 ANSWER 6 OF 6 CAPLUS COPYRIGHT 2005 ACS on STN
AN 1991:402487 CAPLUS
DN 115:2487
TI Cysteine-added **variants** of polypeptides and chemical **modifications** thereof
IN Shaw, Gray; Veldman, Geertruida; Wooters, Joseph
PA Genetics Institute, Inc., USA
SO PCT Int. Appl., 47 pp.
CODEN: PIXXD2
DT Patent
LA English

FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9012874	A2	19901101	WO 1990-US2144	19900419 <--
	WO 9012874	A3	19910110		
	W: AU, CA, JP				
	RW: AT, BE, CH, DE, DK, ES, FR, GB, IT, LU, NL, SE				
	US 5166322	A	19921124	US 1989-341990	19890421 <--
	AU 9055537	A1	19901116	AU 1990-55537	19900419 <--

	EP 469074	A1	19920205	EP 1990-907849	19900419 <--
	EP 469074	B1	19960731		
	R: AT, BE, CH, DE, DK, ES, FR, GB, IT, LI, LU, NL, SE				
	JP 04504801	T2	19920827	JP 1990-507086	19900419 <--
	JP 2557144	B2	19961127		
	EP 668353	A1	19950823	EP 1995-103989	19900419 <--
	R: AT, BE, CH, DE, DK, ES, FR, GB, IT, LI, LU, NL, SE				
	EP 668354	A1	19950823	EP 1995-103990	19900419 <--
	R: AT, BE, CH, DE, DK, ES, FR, GB, IT, LI, LU, NL, SE				
	AT 140969	E	19960815	AT 1990-907849	19900419 <--
	ES 2090132	T3	19961016	ES 1990-907849	19900419 <--
PRAI	US 1989-341990	A	19890421		
	EP 1990-907849	A3	19900419		
	WO 1990-US2144	A	19900419		

AB **Analogs** of polypeptides in which cysteines are substituted for other amino acids or are inserted [cysteine-added **variants** (CAVs)] are prepared by expression of the gene in an heterologous host. CAVs of human interleukin-3 (IL-3), **granulocyte-colony stimulating factor** (**G-CSF**) and eryhthropoietin (EPO) are prepared to improve their therapeutic efficacy. The method comprises **substitution** with or insertion of >1 cysteine residues to the natural proteins and, preferably, deletion of certain N-terminal amino acids and **modification** of the new cysteine sites by coupling of the thiol. More than 15 **analogs** of human IL-3 with modified N-termini, e.g. deletion of Ala-1, and addnl. cysteine residues at positions 3, 6, 8, 10, 12, 100, etc. were prepared by conventional oligonucleotide-mediated site-specific mutations and expression of the genes in animal or microbial hosts. HPLC-purified CAVs of IL-3 were refolded by reacting with a **PEG** derivative e.g. S-pyridyl monomethoxy **PEG** 5000 or maleimido monomethoxy **PEG** 5000. Biol. activities of these CAVs of IL-3 were also observed

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- Search numbers may not be continuous; all searches are represented.
- Click on query # to add to strategy

Search	Most Recent Queries	Time	Result
#8	Search **#7 AND (substitution or variant or lysine)** Field: **Title/Abstract,** Limits: **Publication Date to 2001/01/10**	18:01:15	41
#7	Search **(g-csf OR gscf OR "granulocyte-colony stimulating factor" OR "ganulocyte colony-stimulating factor" OR "ganulocyte colony stimulating factor" OR hg-csf) AND (structure OR analog* OR mutant OR substitution OR mutagenesis)** Field: **Title/Abstract,** Limits: **Publication Date to 2001/01/10**	17:59:59	315
#6	Search **(G-CSF or GSCF or "granulocyte-colony stimulating factor" or "ganulocyte colony-stimulating factor" or "ganulocyte colony stimulating factor" or hg-csf) AND (strucutre or analog* or mutant or substitution or mutagenesis)** Field: **Title/Abstract,** Limits: **Publication Date to 2001/01/10**	17:59:45	225
#5	Search **(G-CSF or GSCF or "granulocyte-colony stimulating factor" or "ganulocyte colony-stimulating factor" or "ganulocyte colony stimulating factor" or hg-csf) AND (PEG or polyethylene)** Field: **Title/Abstract,** Limits: **Publication Date to 2001/01/10**	17:58:33	36
#4	Search **(G-CSF or GSCF or "granulocyte-colony stimulating factor" or "ganulocyte colony-stimulating factor" or "ganulocyte colony stimulating factor" or hg-csf) AND (PEG or polyethylene)** Field: **Title/Abstract**	17:58:10	54
#3	Search **(G-CSF or GSCF or "granulocyte-colony stimulating factor" or "ganulocyte colony-stimulating factor" or "ganulocyte colony stimulating factor" or hg-csf) AND (PEG or polyethylene)**	17:58:02	94
#1	Search **yamasaki[au] AND polyethylene[ti]**	12:57:26	5

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☐	L22	L21 and (PEG or polyethylene) same lysine	694
☐	L21	(granulocyte adj colony adj stimulating adj factor or hg-csf or g-csf or GCSF) same (polyethylene or PEG)	813
☐	L20	(granulocyte adj colony adj stimulating adj factor or hg-csf or g-csf or GCSF) same (polyethylene or PEG or glycos$)	1071
☐	L19	(L1 or l3) and ((glutamine or glutamic adj acid) with 70 or Q70$1 or Glu70$3) same (substitut$ or lysine)	36
☐	L18	(L1 or l3) and (lysine with (16 34 40)or lys16 or lys34 or lys40 or K16 or K34 or K40) same (substitut$ or arginine)	110
		DB=USPT,PGPB,JPAB,EPAB,DWPI; PLUR=YES; OP=OR	
☐	L17	L16 and L4	3
☐	L16	L14 and (granulocyte adj colony adj stimulating adj factor or hg-csf or g-csf or GCSF).clm. and antagonist.clm.	86
☐	L15	L14 and L4	30
☐	L14	(granulocyte adj colony adj stimulating adj factor or hg-csf or g-csf or GCSF) same antagonist	807
☐	L13	L4 and antagonist	49
☐	L11	L10 and L4	37
☐	L10	L2 and (peg or polymer of glycosylation or polyethylene) same (lysine or lys)	1857
☐	L5	L4 and L2	106
☐	L4	L3 and (granulocyte adj colony adj stimulating adj factor or hg-csf or g-csf or GCSF).ab.	250
☐	L3	(granulocyte adj colony adj stimulating adj factor or hg-csf or g-csf or GCSF) same (substitution or mutant or mutation or variant or analog or derivative)	1934
☐	L2	L1 and (polymer or PEG or polyethylene)	6295
☐	L1	(granulocyte adj colony adj stimulating adj factor or hg-csf or g-csf or GCSF) and (substitution or mutant or mutation or variant or analog or derivative)	8207

END OF SEARCH HISTORY